

IN THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1-19. (Cancelled).

20. (New) A synthetic DNA promoter, said promoter comprising at least one of each of the following elements, or functional fragments thereof, in the 5' to 3' direction:

(i) domain II which comprises at least one member selected from the group consisting of subdomain II (a), subdomain II (b), subdomain II (c), subdomain II (d) and domain III, wherein subdomain II (a) is SEQ ID NO: 7, or subdomain II (a) is a functional sequence with at least 50% sequence identity to SEQ ID NO: 7; subdomain II (b) is SEQ ID NO: 8, or subdomain II (b) is a functional sequence with at least 65% sequence identity to SEQ ID NO: 8; subdomain II (c) is SEQ ID NO: 9, or subdomain II (c) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 9; subdomain II (d) is SEQ ID NO: 10, or subdomain II (d) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 10; and domain III is SEQ ID NO: 11, or domain III is a functional sequence with at least 75% sequence identity to SEQ ID NO: 11;

(ii) domain I, which comprises at least one member selected from the group consisting of subdomain I (a), subdomain (b), and subdomain (c), wherein subdomain I (a) is SEQ ID NO: 18, or subdomain I (a) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 18; subdomain I (b) is SEQ ID NO: 19, or subdomain I (b) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 19; and subdomain I (c) is SEQ ID NO: 20, or subdomain I (c) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 20;

(iii) minimal domain (b), wherein minimal domain (b) is SEQ ID NO: 5, or minimal domain (b) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 5;

(iv) minimal domain (a), wherein minimal domain (a) is SEQ ID NO: 2, or minimal domain (a) is a functional sequence at least 75% homologous to SEQ ID NO: 2, or minimal domain (a) is SEQ ID NO: 3, or minimal domain (a) is a functional sequence at least 60% homologous to SEQ ID NO: 3;

(v) region between minimal promoter (a) and a transcription start site context, wherein said region between minimal promoter (a) and said transcription start site context is SEQ ID NO: 12, or said region between minimal promoter (a) and the transcription start site is a sequence with at least 75% sequence identity to SEQ ID NO: 12;

(vi) transcription start site context, wherein said transcription start site context is SEQ ID NO: 4, or said transcription start site context is a functional sequence with at least 50% sequence identity to SEQ ID NO: 4;

(vii) 5' untranslated leader region, wherein said 5' untranslated leader region is SEQ ID NO: 13, or said 5' untranslated leader region is a sequence with at least 75% sequence identity to SEQ ID NO: 13;

(viii) translational initiation codon context, wherein said translational initiation codon context is SEQ ID NO: 14, or said translational initiation codon context is a sequence with at least 75% sequence identity to SEQ ID NO: 14, or said translational initiation codon context is SEQ ID NO: 15, or said translational initiation codon context is a sequence with at least 50% sequence identity to SEQ ID NO: 15; and

(ix) codons that encode four N-terminal amino acids in frame with a nucleic acid sequence of a gene to which said promoter is linked, wherein said N-terminal amino acids correspond to SEQ ID NO. 16.

21. (New) The synthetic DNA promoter of claim 20, wherein subdomain II (a) is SEQ ID NO: 7.

22. (New) The synthetic DNA promoter of claim 20, wherein subdomain II (b) is SEQ ID NO: 8.

23. (New) The synthetic DNA promoter of claim 20, wherein subdomain II (c) is SEQ ID NO: 9.

24. (New) The synthetic DNA promoter of claim 20, wherein subdomain II (d) is SEQ ID NO: 10.

25. (New) The synthetic DNA promoter of claim 20, wherein domain III is SEQ ID NO: 11.

26. (New) The synthetic DNA promoter of claim 20, wherein domain I (a) is SEQ ID NO: 18.

27. (New) The synthetic DNA promoter of claim 20, wherein domain I (b) is SEQ ID NO: 19.

28. (New) The synthetic DNA promoter of claim 20, wherein domain I (c) is SEQ ID NO: 20.

29. (New) The synthetic DNA promoter of claim 20, wherein minimal domain (b) is SEQ ID NO: 5.

30. (New) A synthetic DNA promoter, said promoter comprising at least one of each of the following elements, or functional fragments thereof, in the 5' to 3' direction:

(i) minimal domain (a), wherein minimal domain (a) is SEQ ID NO: 2, or minimal domain (a) is a functional sequence at least 75% homologous to SEQ ID NO: 2, or minimal domain (a) is SEQ ID NO: 3, or minimal domain (a) is a functional sequence at least 60% homologous to SEQ ID NO: 3;

(ii) region between minimal promoter (a) and a transcription start site context, wherein said region between minimal promoter (a) and said transcription start site context is SEQ ID NO: 12, or said region between minimal promoter (a) and the transcription start site is a sequence with at least 75% sequence identity to SEQ ID NO: 12;

(iii) transcription start site context, wherein said transcription start site context is SEQ ID NO: 4, or said transcription start site context is a functional sequence with at least 50% sequence identity to SEQ ID NO: 4;

(iv) 5' untranslated leader region, wherein said 5' untranslated leader region is SEQ ID NO: 13, or said 5' untranslated leader region is a sequence with at least 75% sequence identity to SEQ ID NO: 13; and

(v) translational initiation codon context, wherein said translational initiation codon context is SEQ ID NO: 14, or said translational initiation codon context is a sequence with at least 75% sequence identity to SEQ ID NO: 14, or said translational initiation codon context is SEQ ID NO: 15, or said translational initiation codon context is a sequence with at least 50% sequence identity to SEQ ID NO: 15.

31. (New) The synthetic DNA promoter of claim 30, wherein minimal domain (a) is SEQ ID NO: 2.

32. (New) The synthetic DNA promoter of claim 30, wherein said region between minimal promoter (a) and said transcription start site context is SEQ ID NO: 12.

33. (New) The synthetic DNA promoter of claim 30, wherein said transcription start site context is SEQ ID NO: 4.

34. (New) The synthetic DNA promoter of claim 30, wherein said 5' untranslated leader region is SEQ ID NO: 13.

35. (New) The synthetic DNA promoter of claim 30, wherein said translational initiation codon context is SEQ ID NO: 14.

36. (New) A method for chemically synthesizing a synthetic DNA promoter that directs the expression of a gene at a desired level in an organism, which comprises:

- a) classifying genes from a DNA sequence database into highly and lowly expressed genes,
- b) aligning nucleic acid sequences of said genes surrounding transcription and/or translation regulatory regions that determine expression of said genes,
- c) identifying conserved domains within said regulatory regions,
- d) designing a synthetic promoter comprising the placement of conserved domains identified in step c) in a logical arrangement to achieve the desired level of expression of a reporter or target gene, and
- e) chemically synthesizing a said synthetic DNA promoter designed in step d).

37. (New) The method of claim 36, wherein said step e) comprises synthesizing said synthetic DNA promoter by synthesizing overlapping oligonucleotides and assembling said oligonucleotides into a double stranded DNA synthetic DNA promoter.

38. (New) The method of claim 36, further comprising cloning said promoter 5' of a reporter gene or a target gene in a suitable vector selected for expression.

39. (New) A method for testing the level of expression of a gene in a plant comprising:

a) providing a test construct comprising a target gene or reporter gene linked in proper orientation with the synthetic DNA promoter of SEQ ID NO. 1,

b) providing plant protoplasts,

c) transforming the plant protoplasts with the test construct using polyethylene glycol (PEG) mediated transformation, and

d) performing a transient GUS assay to compare the expression of the test construct with that of the same target gene or reporter gene under the control of a natural CaMV 35S promoter showing the desired level of activity.

40. (New) The method of claim 39, wherein the plant is tobacco, cotton, cabbage or potato.

41. (New) The method of claim 39, wherein the protoplasts are derived from a plant tissue.

42. (New) The method of claim 41, wherein the plant tissue is root, shoot, leaf or storage tissue.

43. (New) The method of claim 39, wherein the target gene is uidA.

44. (New) A method for testing the level of expression of a gene in a plant comprising:

a) providing a test construct comprising a target gene or reporter gene linked in proper orientation with the synthetic DNA promoter of SEQ ID NO. 1,

b) providing plant protoplasts,

b) transforming the plant protoplasts with the test construct using biolistic-mediated transformation, and

c) performing a transient GUS assay to compare the expression of the test construct with that of the same target gene or reporter gene under the control of a natural CaMV 35S promoter showing the desired level of activity.

45. (New) The method of claim 44, wherein the protoplasts are derived from a plant tissue.

46. (New) The method of claim 45, wherein the plant tissue is root, shoot, leaf or storage tissue.

47. (New) The synthetic promoter of claim 20, further comprising codons that encode four N-terminal amino acids in frame with a nucleic acid sequence of a gene to which said promoter is linked, wherein said N-terminal amino acids correspond to SEQ ID NO. 16.

48. (New) The synthetic promoter of claim 30, further comprising codons that encode four N-terminal amino acids in frame with a nucleic acid sequence of a gene to which said promoter is linked, wherein said N-terminal amino acids correspond to SEQ ID NO. 16.

49. (New) A synthetic DNA promoter produced by the following steps:

- a) classifying genes from a DNA sequence database into highly and lowly expressed genes,
- b) aligning nucleic acid sequences of said genes surrounding transcription and/or translation regulatory regions that determine expression of said genes,
- c) identifying conserved domains within said regulatory regions,
- d) designing a synthetic promoter comprising the placement of conserved domains identified in step c) in a logical arrangement to achieve the desired level of expression of a reporter or target gene, and
- e) chemically synthesizing the synthetic DNA promoter designed in step d).

50. (New) The synthetic DNA promoter of claim 49, wherein said step e) comprises synthesizing said synthetic DNA promoter by synthesizing overlapping oligonucleotides and assembling said oligonucleotides into a double stranded DNA synthetic DNA promoter.

51. (New) The synthetic DNA promoter of claim 50, wherein the conserved domains are at least one member selected from the group consisting of:

(i) domain II, which comprises at least one member selected from the group consisting of subdomain II (a), wherein subdomain II (a) is SEQ ID NO: 7, or subdomain II (a) is a functional sequence with at least 50% sequence identity to SEQ ID NO: 7, subdomain II (b), wherein subdomain II (b) is SEQ ID NO: 8, or subdomain II (b) is a functional sequence with at least 65% sequence identity to SEQ ID NO: 8, subdomain II (c), wherein subdomain II (c) is SEQ ID NO: 9, or subdomain II (c) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 9, subdomain II (d), wherein subdomain II (d) is SEQ ID NO: 10, or subdomain II (d) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 10, and domain III, wherein domain III is SEQ ID NO: 11, or domain III is a functional sequence with at least 75% sequence identity to SEQ ID NO: 11;

(ii) domain I, which comprises at least one member selected from the group consisting of subdomain I (a), wherein subdomain I (a) is SEQ ID NO: 18, or subdomain I (a) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 18, subdomain I (b), wherein subdomain I (b) is SEQ ID NO: 19, or subdomain I (b) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 19, and subdomain I (c), wherein subdomain I (c) is SEQ ID NO: 20, or subdomain I (c) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 20;

- (iii) minimal domain (b), wherein minimal domain (b) is SEQ ID NO: 5, or minimal domain (b) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 5;
- (iv) minimal domain (a), wherein minimal domain (a) is SEQ ID NO: 2, or minimal domain (a) is a functional sequence at least 75% homologous to SEQ ID NO: 2;
- (v) region between minimal promoter (a) and a transcription start site context, wherein said region between minimal promoter (a) and said transcription start site context is SEQ ID NO: 12, or said region between minimal promoter (a) and the transcription start site is a sequence with at least 75% sequence identity to SEQ ID NO: 12;
- (vi) transcription start site context, wherein said transcription start site context is SEQ ID NO: 4, or said transcription start site context is a functional sequence with at least 50% sequence identity to SEQ ID NO: 4;
- (vii) 5' untranslated leader region, wherein said 5' untranslated leader region is SEQ ID NO: 13, or said 5' untranslated leader region is a sequence with at least 75% sequence identity to SEQ ID NO: 13; and
- (viii) translational initiation codon context, wherein said translational initiation codon context is SEQ ID NO: 14, or said translational initiation codon context is a sequence with at least 75% sequence identity to SEQ ID NO: 14.

52. (New) The synthetic promoter of claim 49, further comprising codons that encode four N-terminal amino acids in frame with a nucleic acid sequence of a gene to which said promoter is linked, wherein said N-terminal amino acids correspond to SEQ ID NO. 16.